Supporting information for Fruman *et al*. (December 26, 2001) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.012605099.

Materials and Methods

Data Analysis. Reliability of microarray data is limited by several factors. These include the discriminatory ability of the oligomer probes, as well as variability in the quality of the chips, the hybridization, and the biological sample. To minimize the first three problems, we analyzed raw hybridization data using D-CHIP software (www.dchip.org) (1). This program identifies unreliable "outlier" probe pairs and excludes their contribution to the calculation of the expression value (also known as the "average difference") for each gene. Probe sets affected by scratches or hybridization artifacts are also identified. Using D-CHIP to determine the average difference has been shown to provide a more reliable measure of gene expression than the corresponding values calculated by Affymetrix software (1). Data from each subarray (Mu11KsubA, Mu11KsubB, U74Bv2, U74Cv2) were normalized to the chip with the median hybridization signal. Probe sets flagged by D-CHIP were treated as absent data in subsequent analyses.

To limit the influence of biological variability and sample handling error, it is important to analyze replicate samples and apply rigorous statistical methods (2). Therefore, we purified B cells from pooled splenocytes of several mice before splitting the cells into treatment groups. In addition, each data point was replicated two to three times, and all hybridization data were modeled as a group in D-CHIP. The resulting expression data were analyzed by using Cybert (www.genomics.uci.edu), a Web-based program developed by Long and colleagues that uses a Bayesian framework for analysis of variance (3, 4). In brief, Bayesian analysis calculates the error of measurements on the basis of both the empirical variance and the variance of genes with similar expression levels. It has been shown that this approach improves statistical inferences made from microarray data, compared to the use of other statistical tests or arbitrary fold-change cutoffs (3, 4). In pairwise comparisons of experimental conditions, Cybert was used to determine probability (*P*) values, on the basis of *t*-test distribution, for expression differences for each gene. The Bayesian factor (confidence value) was set at 6 with a sliding window of 101 genes, with a lower cutoff of 1 (3, 4). Genes that did not have a positive expression value for at least two replicates were excluded from the analysis. The output of Cybert analysis includes a *P* value even when data are supplied in duplicate. The output files were sorted by *P* value, and genes measured with *P* values less than 0.01 were graphed, clustered, and inspected by using GeneSpring software (Silicon Genetics). Expression data were further normalized to each gene within GeneSpring to plot all the genes on a similar scale. K-means clustering was performed within GeneSpring, by using a Pearson correlation as the similarity metric, a maximum iteration number of 100, and a filter to exclude genes whose raw expression index did not reach a mean value of 500 in at least one condition.

Oligonucleotide Sequences. Primer sequences for Q-PCR were: Gadd153, 5'-AAACGAAGAGGAAGAATCAAA-3' and 5'-TCCTGGGCCATAGAACTC -3';

CD62L, 5'-TCCTGCATTGAGTTTTAGTTTT-3' and 5'-GAGAGTGGGTTGTGTTATCATT-3'; Cyclin D2, 5'-GCCAAGATCACCCACACT-3' and 5'-GCTGCTCTTGACGGAACT-3'; CD2, 5'-CAAGGGGAAACACTACTCAA-3' and 5'-GGTTTATCGCCTCACACTT-3'; Gfi-1, 5'-ATTATTGGCCGCAGTTATC-3' and 5'-CAGGCTCTAGCTATGTTGAAGT-3'; Enx-1, 5'-CTGATGCCCTGAAGTATGTG-3' and 5'-AGAAGGGGAAGAGGTAGTAGATG-3'; glutamic acid decarboxylase, 5'-AGATAGCCCTGAGCGACGAGAAA-3' and 5'-GTGGCGGCTGGGTTAGAGATGA-3'; Stat4, 5'-TCCCCTCTGTTTTTATCCCCATTT-3' and 5'-TGTCCGTTTGCACCGTCATTC-3'; β-actin, 5'-AGGCCAACCGTGAAAAG-3' and 5'-GCGTGAGGGAGAGCATAG-3'.

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